TRADE SECRET

STUDY TITLE: H-28548: Absorption, Distribution, Metabolism, and Elimination in the Rat

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines
OPPTS 870.7485 (1998)

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ORIGINAL REPORT COMPLETED: November 3, 2010

REPORT REVISION 1 COMPLETED: April 21, 2011

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LABORATORY PROJECT ID: DuPont-18405-1017

WORK REQUEST NUMBER: 18405

SERVICE CODE NUMBER: 1017

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices, except for the item documented below. The item listed does not impact the validity of the study.

1. Qualitative analysis of urine samples for structure confirmation and elucidation was conducted on a non-GLP Liquid Chromatography/Mass Spectrometry (LC/MS) system. However, the identity of the parent analyte, the only analyte detected, was confirmed in urine samples using the test substance H-28548, which had a matching nominal mass-to-charge (m/z) ratio of approximately 329.

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Director: _____ 21-APR-2011
William J. Fasano, Sr., B.S. Date
Senior Research Toxicologist

Sponsor: _____ _____
Sponsor Representative Date

QUALITY ASSURANCE STATEMENT

Work Request Number: 18647
Service Code Number: 1017

Key inspections for the above referenced study were completed by the Quality Assurance Unit of DuPont Haskell and the findings were submitted on the following dates:

Audit Dates	***Date Reported to Study Director***	***Date Reported to Management***
Protocol:		
March 17, 2010	March, 17, 2010	March, 17, 2010
Conduct:		
March 29, 2010	March 29, 2010	March 29, 2010
May 24, 2010	May 24, 2010	May 24, 2010
Report/Records:		
October 04-07,13, 2010	October 13, 2010	October 14, 2010
Sponsor Edits 1:		
October 28, 2010	October 28, 2010	October 28, 2010
Report Revision 1:		
April 11, 2011	April 11, 2011	April 11, 2011

Reported by: _____ 19 Apr 2011
Antonio Pedulla — Date
Quality Assurance Auditor

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

LC/MS/MS Quantitation by: FOR MPM — 20-APR-2011
Michael P. Mawn, Ph.D.
Senior Research Chemist
Date

LC/MS Metabolite ID by: — 21-APR-2011
Timothy A. Snow, Ph.D.
Senior Research Chemist
Date

Reviewed and Approved by: — 19-APR-2011
Gary W. Jepson, Ph.D.
Manager
Date

Issued by Study Director: — 21-APR-2011
William J. Fasano, Sr., B.S.
Senior Research Toxicologist
Date

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STUDY INFORMATION

Substance Tested:	• HFPO Dimer Acid Ammonium Salt • 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt • 62037-80-3 (CAS Number) • H-28548
Haskell Number:	28548
Composition:	Proprietary
Purity:	84%
Physical Characteristics:	Clear and colorless liquid
Stability:	The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
Study Initiated/Completed:	March 16, 2010 / (see report cover page)
Experimental Start/Termination:	March 23, 2010 / July 1, 2010
In-Life Initiated/Completed:	March 23, 2010 / March 30, 2010
Notebook Number(s):	E-114321-AH, E-98524-GF, E-114321-AL

REASON FOR REVISION 1

The elimination half-life ($T_{1/2}$) for H-28548 in male and female rats, following a single oral dose at 30 mg/kg, was estimated and reported.

SUMMARY

The absorption, distribution, metabolism, and elimination of H-28548 were investigated in the Sprague-Dawley rat. H-28548 was administered in water to 5 male and 5 female rats as a single oral dose at a target dose level of 30 mg H-28548/kg bodyweight (bw) and a dose volume of 4 mL/kg bw. Rats were housed individually in glass metabolism units and urine and feces were collected on dry ice predose and postdose at 0-6 hours, 6-12 hours, 12-24 hours, and every 24 hours until 168 hours post-dose. At 168 hours post-dose, rats were asphyxiated by exposure to carbon dioxide and then sacrificed by exsanguination. H-28548 was quantitated in urine, feces, and cagewash by liquid chromatograpy tamdem mass spectrometry (LC/MS/MS). Urine samples were further evaluted by LC/MS to confirm the identity of the parent analyte and determine if H-28548 was eliminated metabolized or unmetabolized.

Following oral administration of H-28548 in water, 96.6% ± 1.43% and 94.6% ± 8.57% of the administered dose was accounted for in urine (0-12 hours) from male and female rats, respectively. At the conclusion of the study (168 hours post-dose), the total accumulated amount of H-28548 detected in urine was 103% ± 2.73% and 99.8% ± 6.41% of the administered dose for male and female rats, respectively.

Elimination of H-28548 via urine was rapid and accounted for a majority of the administered dose for both male and female rats; negligible levels of H-28548 detected in feces from male (1.35% ± 1.05%) and female rats (0.85% ± 0.58%), were likely contamination from urine.

Cagewash, which is composed of dried excreta (urine and feces), accounted for 0.98% ± 0.52% and 5.03% ± 5.14% of the administered dose for male and female rats, respectively.

Following oral dosing with H-28548 in water and a 168 hour post-dose collection period, 105.3% ± 2.19% and 105.7% ± 1.42% of the administered dose was recovered from male and female rats, respectively.

Samples of urine evaluated using LC/MS were found to contain only the parent substance, H-28548. This finding, taken with the complete recovery of the administered dose in urine, confirms that H-28548 was rapidly absorbed and eliminated unmetabolized following oral dosing in the rat.

The elimination half-life ($T_{1/2}$) for H-28548 in male and female rats, following a single oral dose at 30 mg/kg, was estimated to be 3 and 8 hours, respectively.

INTRODUCTION

The data from this study provides basic information on the absorption, distribution, metabolism, and elimination (ADME) of H-28548 following oral dosing in the rat.

OBJECTIVE

The objective of this study was to determine the ADME of H-28548 in the rat following a single oral dose of H-28548 in water. Use of a non-radiolabeled test substance for determining a material balance and metabolite identification is justified based on results from an *in vitro* metabolism experiment with rat hepatocytes and rat oral and rat and monkey intravenous dose kinetic studies, which suggests that H-28548 is not metabolized and is eliminated rapidly.[1,2,3,4]

ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals (NRC 1996). All studies conducted by or for DuPont Haskell adhere to the following principles:

- The sponsor and/or the study director ensures that the study described in this report does not unnecessarily duplicate previous experiments, and is in compliance with the DuPont Policy on Animal Testing.
- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals.
- DuPont Haskell policy is that animals experiencing severe pain or distress that cannot be relieved are painlessly euthanized, as deemed appropriate by the veterinary staff and study director or appropriate designee.
- Methods of euthanasia used during this study were in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia.
- DuPont Haskell is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guideline:

- U.S. EPA, OPPTS 870.7485. Metabolism and Pharmacokinetics, Health Effects Test Guidelines (1998)

B. Test Substance

The test substance (CAS registry number 62037-80-3) was supplied by the sponsor and assigned Haskell number 28548.

C. Test System

Male and female Crl:CD(SD) rats were obtained from Charles River Laboratories, Inc. (Raleigh, North Carolina, U.S.A.).

The Sprague-Dawley rat was chosen for this study because of the extensive experience with this strain and its suitability with respect to longevity, sensitivity, and low incidence of spontaneous diseases. Furthermore, the Sprague-Dawley rat has been used previously for toxicokinetic and toxicity testing of this chemical.

Each animal was assigned a unique identification number to be used throughout the study. The last 3 digits of the animal identification number were marked on the tail of each animal in indelible ink.

D. Animal Husbandry

1. Housing

During the pretest period, animals were housed individually in solid bottom caging with bedding. Animals were moved to metabolism units for the in-life phase of the study.

2. Environmental Conditions

Animal rooms were maintained at a temperature of 18-26ºC (64-79ºF) and a relative humidity of 30-70%. Animal rooms were artificially illuminated (fluorescent light) on an approximate 12 hour light/dark cycle.

3. Feed and Water

All animals were provided tap water *ad libitum* and fed PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 *ad libitum*. When housed in metabolism units, feed was supplied as ground chow.

4. Animal Health and Environmental Monitoring Program

As specified in the DuPont Haskell animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.

- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

E. Pretest Period

Upon arrival at DuPont Haskell, all rats were housed in quarantine. The rats were:

- quarantined for at least 6 days.
- identified temporarily by cage identification.
- weighed at least 3 times during quarantine and once prior to dosing.
- observed with respect to weight gain and any gross signs of disease or injury.

The animals were released from quarantine by the laboratory animal veterinarian or designee based on body weights and clinical signs.

F. Assignment to Groups

Animals were selected for use on study based on adequate body weight gain and freedom from any clinical signs of disease or injury. The weight variation of selected animals by sex was less than 4% of the mean weight.

Each animal was assigned an animal number and a cage identification number. The animal number and cage identification number were both included on the cage label.

At study start, the animals were at least 8 weeks old.

G. Dose Preparation, Analysis, and Rates

The test substance was prepared for administration by oral gavage. This route was chosen because it is most commonly used for toxicity studies with H-28548.

H-28548 was weighed into a vial (approximately 178.5 mg) and mixed with deionized water (20 mL). The dose solution was prepared at a nominal concentration of 7.5 mg H-28548/mL (adjusted for purity, 84%), with a target dose level of 30 mg/kg body weight (bw) and a dose volume of 4 mL/kg bw. The dose level was chosen based on the results of the 28-day daily oral

dosing study in rats, where the no-observed-adverse-effect level (NOAEL) was 30 and 300 mg/kg/day for males and females, respectively.[5]

The dosing solution was prepared prior to the day of use and was stored refrigerated at 1-10°C prior to dosing.

H. In-Life Phase

1. Material Balance and Tissue Distribution

The conduct of this study was designed to comply with the Tier 1 requirements of U.S. EPA, OPPTS 870.7485 - Metabolism and Pharmacokinetics, Health Effects Test Guidelines (1998).

Rats were housed individually in glass metabolism units and fasted for approximately 16 hours prior to dosing. Food was returned approximately 2 hours post-dose.

Five male and 5 female rats were administered H-28548 at a nominal target of 30 mg H-28548/kg bw. Two male and 2 female rats were each administered dose vehicle (deionized water at 4 mL/kg bw) for collection of control excreta and tissue samples. Rats were returned to individual metabolism units following dosing.

Urine and feces were collected on dry ice predose and at 0-6 h, 6-12 h, 12-24 h, and every 24 hours until 168 hours post dose. Evidence supporting a lack of metabolism of H-28548 in rat hepatocytes and rat oral dose administration studies, precluded the necessity for a radiolabeled form of H-28548 and collection of expired air.

At the end of the experiment (168 hours post dose), rats were killed by CO_2 asphyxiation followed by exsanguination. The following tissues (Tier 1) were collected:

liver
fat
G.I. tract (and contents)
kidney
spleen
whole blood
residual carcass

After collection, these samples were stored at approximately ≤-10°C.

Over the course of the experiment, residual feed was collected into a single container and stored refrigerated at 1-10°C. Cages were rinsed with deionized water, which was collected into a single container. Cage wash was stored at room temperature and/or refrigerated at 1-10°C.

I. Quantitation of H-28548

1. Sample Receipt

The dose solution, urine, feces, and cage wash samples were received and stored at approximately -20°C by the analytical laboratory upon receipt and when not in use.

2. Sample Preparation Procedure (dose solution and urine samples)

The frozen samples were thawed to room temperature and mixed briefly before sampling. A pipette was used to transfer 25 µL of sample into an empty HPLC vial, and the sample weight was recorded to the nearest 0.0001 gram. The pipette was then used to add 975 µL of HPLC grade water, and mixed. The initial sample preparation dilution factor = 1/sample weight (g). Additional sample dilutions were performed with HPLC grade water to ensure that the sample peak area results were within the calibration curve limits. Quality control fortification samples were also prepared at low, mid and high levels in control urine, and prepared for analysis using the same procedure.

3. Sample Preparation Procedure (cage wash samples)

The frozen cage wash samples were thawed to room temperature and mixed briefly before sampling. A pipette was used to transfer 200 µL of sample into an empty HPLC vial, and the sample weight was recorded to the nearest 0.0001 gram. The pipette was then used to add 800 µL of HPLC grade water, and mixed. The initial sample preparation factor = 1/sample weight (g).

4. Sample Preparation Procedure (feces samples)

The frozen feces samples submitted in 50-mL conical polypropylene centrifuge tubes were thawed to room temperature. HPLC grade water was added to the 40-mL mark, and the weight of water added was recorded to the nearest 0.1 gram. Five ball bearings (5/32" diameter) were added to the sample tubes and sealed. The samples were homogenized using a Genogrinder for 5 minutes at 1400 strokes/minute (SPEX CertiPrep Genogrinder 2000, Metuchen, New Jersey U.S.A.). After homogenization, the samples were placed in a refrigerator for overnight extraction. After overnight extraction the samples were shaken to mix and centrifuged for 10 minutes at 4150 rpm at 20ºC. Approximately 1.5 mL of supernatant was added to a 1.7 mL microcentrifuge tube and further centrifuged for 15 minutes at 14,000 rpm and 20 ºC. A syringe filter (PALL Acrodisc - 25 mm with 0.2 µm Nylon Membrane) was then used to filter approximately 1 mL supernatant into a HPLC vial for analysis. The preparation factor = (H_2O weight (g) + feces weight (g)) / feces weight (g). Additional sample dilutions were performed with HPLC grade water to ensure that the sample peak area results were within the calibration curve limits. Quality control fortification samples were also prepared at low, mid and high levels using 2 grams of control feces, and prepared for analysis using the same procedure.

5. Stock Solutions and Calibration Standards

A stock solution of H-28548 was prepared in HPLC grade water. The stock solution was diluted with HPLC grade water to prepare calibration standards at 0, 2.50, 5.00, 12.5, 25.0, 62.5, 156, and 250 ng/mL levels.

6. Instrument and Conditions

The prepared samples were analyzed by LC/MS/MS using the following conditions:

Method 1	Quantitation of H-28548 in urine, feces, and cagewash
HPLC Instrument:	Agilent Model 1100

HPLC Parameters:

Column (Urine, dose solution, and cage wash):	Zorbax SB-C8; 2.1x100 mm with 3.5 micron particle size
Column (Feces)	Zorbax SB-C8; 2.1x30 mm with 3.5 micron particle size
Mobile Phase:	A: 0.15% acetic acid in HPLC grade water B: 0.15% acetic acid in acetonitrile
Column Temperature:	35 °C
Injection Volume:	5 µL urine, dose and cage wash samples 2 µL for feces samples

HPLC Gradient (Urine, dose solution, and cage wash samples)	Total Time (min)	Flow Rate (µL/min)	A (%)	B (%)
	0.00	400	65.0	35.0
	5.00	400	65.0	35.0

HPLC Gradient (Feces samples)	Total Time (min)	Flow Rate (µL/min)	A (%)	B (%)
	0.00	400	95.0	5.0
	2.00	400	95.0	5.0
	2.10	400	70.0	30.0
	4.50	400	50.0	50.0
	6.00	400	5.0	95.0
	9.00	400	5.0	95.0
	9.10	400	95.0	5.0
	11.0	400	95.0	5.0

MS Parameters:

Ion Source:	Turbo Spray, Negative Ion
Temperature (TEM):	120ºC
Dwell	250 msec

Curtain Gas Flow (CUR):	10.0				
GS1:	25				
GS2:	25				
IonSpray (IS) Voltage:	-4500				
CAD	6.00				
EP	-10.0				
Quadrupole Resolution:	Quad. 1: Unit				
	Quad. 3: Unit				
MRM Settings	Q1 Mass	Q3 Mass	DP	CE	CXP
H-28548	329.0	285.00	-20.0	-6.0	-7.0

7. Quantitation

The samples, calibration standards, and fortification quality control plasma samples were analyzed by LC/MS/MS. The calibration standard curve was generated by regression analysis using the chromatographic peak areas of the calibration standard solutions. The peak areas for the study samples and fortification QC samples were compared to the calibration standard curve to determine the concentration of the analyte. Any samples with peak areas above the upper calibration standard were diluted to ensure that the peak areas were within the calibration curve.

J. Identification of Metabolites

Samples of urine were pooled across animals for a given time interval where the mean percent of the administered dose (by sex) was ≥5% (males: 0-6, 6-12 and 12-24 hours; females: 0-6 and 6-12 hours); feces extract samples were not pooled since the total mean percent of dose for each collection interval (by sex) was <5% of the administered dose.

Samples of pooled urine (25 µL) were diluted to 500 µL with Nanopure water prior to analysis. Samples of the diluted urine (20 µL) were qualitatively screened by LC/HRMS for metabolites. Retention time and mass spectral confirmation of the parent was performed by spiking control urine with approximately 40 ppm (v/v) of the test material (H-28548) and analyzing the spiked sample using the identical method for the study samples (Method 1).

1. Liquid Chromatography/Mass Spectrometry (LC/MS)

Method 2	Qualitative LC/MS Confirmation and Structural Elucidation of metabolites in urine
HPLC/MS System:	Agilent 1100 HPLC with column thermostat and binary pump, autosampler, variable wavelength detector (S/N DE63058654 - Agilent Inc., Little Falls, Delaware, U.S.A.). Thermo-Fisher OrbiTrap FT-MS (S/N 1016B - Thermo-Fisher Scientific Inc., San Jose, California, U.S.A.). The associated computer is loaded with Thermo-Fisher Xcaliber Software (v 2.0.7)
HPLC Conditions:	
Column:	Agilent Zorbax SB-C18 column (2.1 x 150 mm) 3.5 µm particle size

Column Temperature:	25°C
Solvent A:	0.10% Acetic Acid in HPLC grade water
Solvent B:	0.10% Acetic acid in acetonitrile

Gradient:

Time (min)	A (%)	B (%)
0.0	98.0	2.0
20.00	0.0	100.0
25.00	0.0	100.0
25.10	98.0	2.0
30.00	98.0	2.0

Flow Rate:	0.30 mL/min
Run Time:	30.00 min
Injection Volume:	20 µL
UV Wavelength:	190-400 nm

MS Conditions:

Ionization Mode:	Electrospray negative ion
Source Voltage:	3.6 kV
Capillary Temperature:	330°C
Tube Lens voltage:	140 V
Source Current:	100 µA
Data Acquisition Function:	Full Scan = 120-1000 Da (Profile mode), Mass Resolution = 30,000

Daughter Scans (Da)

Identity	Daughters of	Start Mass	End Mass
H-28548	329	90	500

Collision Energy:	25 V daughter ion scan only
Scan Time	Full scan 0.95 sec/scan; Daughter ion scan 0.3 sec/scan
Collision Gas and Pressure:	Argon at 0.000602 mbar

2. Data processing

All chromatograms were screened for differences (chromatographic peaks) in control versus H28548-dosed urine samples using IntelliExtract™; v. 12.0.1 (ACD, Toronto, Ontario, Canada) control-sample comparison software.

STATISTICAL AND DATA ANALYSIS

Group data were represented as a mean ± SD.

The elimination half-life ($T_{1/2}$; time in hours to elimination of ≥50% of the administered dose) for H-28548 in male and female rats was estimated by interpolation of (mean) cumulative urinary excretion data from 0 to 168 hours using Origin v7.0220 (OriginLab Corporation, Northhampton, Massachusetts, USA). The clearance time (CL_{time}), the time to elimination of

≥98.4%, a value mathematically equal to 6 half-lives of the administered dose, was determined from the interpolated data and $T_{1/2}$ calculated ($Cl_{time} \div 6$).

RESULTS AND DISCUSSION

A. Quantitation of H-28548 by LC/MS/MS

(Tables 1-2, Figures 1-3)

1. Calibration Standard Curve

A calibration curve for H-28548 is shown in Figure 1. The curve was generated based on resulting peak areas of the H-28548 analyte using a quadratic equation, and 1/x weighing.

2. Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by comparing the peak-to-peak noise in chromatograms of control matrix versus the signal of the lowest level calibration standard. The initial LOD was calculated as 3 times the concentration equivalent of the mean noise level. The initial LOQ was based on the lowest calibration standard concentration, which had at least a 10x signal-to-noise ratio. For a sample preparation factor of 1x the initial urine and cage wash sample LOD was 0.1 ng/g and for feces the initial LOD was 0.4 ng/g. For a sample preparation factor of 1x the urine, cage wash, and feces matrices all have an initial LOQ of 2.5 ng/g. The final LOD and LOQ for each sample was determined by multiplying the initial values by the sample preparation factor.

Example LOD & LOQ Calculation: Urine sample from animal 001M, 120 hour time point

- 25 µL aliquot sample weight (g) = 0.0279 g
- Sample Preparation Factor = 1 / 0.0279 = 35.8
- Final LOD for this sample = 0.1 ng/g x 35.8 = 4 ng/g (reported to 1 significant digit)
- Final LOQ for this sample = 2.5 ng/g x 35.8 = 89.5 ng/g (reported to 3 significant digits)

Example LOD & LOQ Calculation: Feces sample from animal 001M, 120 hour time point

- Water Extraction Weight = 25.3 g. Feces weight = 14.21 grams
- Sample Preparation Factor = (25.3(g) + 14.21 (g)) / 14.21 (g) = 2.78
- Final LOD for this sample = 0.4 ng/g x 2.78 = 1 ng/g (reported to 1 significant digit)
- Final LOQ for this sample = 2.5 ng/g x 2.78 = 6.95 ng/g (reported to 3 significant digits)

Example LOD & LOQ Calculation: Cage wash sample from animal 001M, 168 hour time point

- 200 µL aliquot sample weight (g) = 0.2000 g
- Sample Preparation Factor = 1 / 0.2000 = 5.00

- Final LOD for this sample = 0.1 ng/g x 5.00 = 0.5 ng/g (reported to 1 significant digit)
- Final LOQ for this sample = 2.5 ng/g x 5.00 = 12.5 ng/g (reported to 3 significant digits)

None of the predose urine or feces samples had detectable levels of H-28548.

3. Chromatographic Results (urine, cage wash, and dose samples)

H-28548 eluted as a well-resolved peak with a retention time of approximately 2.4 minutes. An example chromatogram for the lowest calibration standard at 2.5 ng/mL is shown in Figure 2a. An example chromatogram of a urine control matrix sample is shown in Figure 2b (H-28548 was not detected). A low level fortification quality control (QC) sample is shown in Figure 2c, which was fortified at a level of 400 ng/g, and had a preparation factor of 40x. A 24-hour urine sample from animal 001M, which had a total dilution factor of 1540x is shown in Figure 2d. The final concentration for this sample was 34700 ng/g.

4. Chromatographic Results (feces samples)

H-28548 eluted as a well-resolved peak with a retention time of approximately 5.5 minutes. An example chromatogram for the lowest calibration standard at 2.5 ng/mL is shown in Figure 3a. An example chromatogram of a feces control matrix sample is shown in Figure 3b (H-28548 was not detected). A low level fortification quality control (QC) sample is shown in Figure 3c, which was fortified at a level of 250 ng/g, and had a preparation factor of 20x. A 12 hour feces sample from animal 001M, which had a total dilution factor of 336x is shown in Figure 3d. The final concentration for this sample was 2750 ng/g.

5. Fortification QC Sample Results

The average QC fortification results for the urine matrix are provided in Table 1. The average recoveries for the low level, mid level, and high level fortification standards ranged from 98-99%. The associated coefficient of variation (CV) ranged from 1-2% and demonstrates acceptable method performance.

The average QC fortification results for the feces matrix are provided in Table 2. The average recoveries for the low level, mid level, and high level fortification standards ranged from 85-91%. The associated CV ranged from 3-6% and demonstrates acceptable method performance.

B. Dose Formulation Concentration, Animal Body Weights, Dosing Information

(Table 3, Appendices A-B)

The concentration of H-28548 in the dose solution, as confirmed by LC/MS, was 6.82 mg H-28548/mL, which was approximately 91% of the nominal target (7.5 mg H-28548/mL).

At study initiation (day of dosing), males weighed 247.8 g ± 8.15 g and females weighed 181.1 g ± 4.23 g; the calculated dose rate for male (27.4 ± 0.17 mg/kg bw) and female rats (27.2 ± 0.16 mg/kg bw) were within 10% of the nominal target (30 mg/kg bw).

C. Urine Data

(Table 4, Figure 4, Appendix C)

Following oral administration of H-28548 in water, 96.6% ± 1.43% and 94.6% ± 8.57% of the administered dose (0-12 hours) was accounted for in urine from male and female rats, respectively.

At the conclusion of the study (168 hours post-dose), the cumulative amount of H-28548 detected in urine was 103% ± 2.73% and 99.8% ± 6.41% for male and female rats, respectively.

Elimination of H-28548 via urine was rapid and accounted for the administered dose for both male and female rats.

D. Feces Data

(Table 5, Figure 5, Appendix D)

Following oral administration of H-28548 in water, the cumulative amount of H-28548 detected in feces over the entire collection period (0-168 hours) was 1.35% ± 1.05% and 0.85% ± 0.58% for male and female rats, respectively.

The negligible amount of H-28548 detected in feces was likely contamination from of urine. Given the high levels of H-28548 in urine, and the design of the urine/feces collection system of the metabolism units, feces likely became contaminated with small amounts of urine when contacting surfaces in transit to the feces collection vessel.

E. Material Balance

(Table 6, Figure 6, Appendices E-F)

Following oral dosing with H-28548 in water and a 168 hour post-dose collection period, 105.3% ± 2.19% and 105.7% ± 1.42% of the administered dose was recovered from male and female rats, respectively.

Of the total H-28548 recovered, the majority of administered dose was account for in urine from both males (103.0% ± 2.73%) and females (99.8% ± 6.41%); lesser amounts of H-28548 were accounted for in feces (male = 1.35% ± 1.05%; female = 0.85% ± 0.58%). Cagewash, which is composed of dried excreta (urine and feces) accounted for 0.98% ± 0.52% and 5.03% ± 5.14% of the administered dose for male and female rats, respectively.

The carcass and residual feed were not analyzed for H-28548 because analysis of urine, feces and cagewash accounted for the majority of administered dose with an overall recovery of 100% ± 10%.

F. Metabolite Identification

(Figures 7-9)

H-28548 was detected in its anionic form by negative ESI mass spectrometry. A representative reconstructed chromatogram of ions characteristic of H-28548 (parent) for the 6 hour female dosed rat urine sample and control urine fortified with the H-28548 test substance is shown in Figure 7.

The LC/MS mass spectrum of H-28548 in urine shows a significant amount of its proton bound dimer (m/z 658.943 Da) and sodium bound dimer (m/z 680.923 Da) (Figure 8); the dimer and the sodium dimer were created in the MS system and were not present in the sample itself. The molecular anion (m/z 328.968) was observed in both urine from a rat dosed with H-28548 and the urine fortified with the test substance H-28548, but at a low intensity relative to the dimer adducts. These dimers are not to be confused with a covalent dimer, such as the HFPO acid dimer parent, but are charged dimers sometimes formed, in-source, as a result of the desolvation and ionization processes necessary to be observed by electrospray ionization mass spectrometry.

The daughter ion mass spectra of the parent ion 328.97 Da for urine from a rat dosed with H-28548 and urine fortified with the H-28548 test substance shows the same 2 characteristic fragment ions at m/z 284.977, the loss of CO_2 and 169.989, $[C_3F_7]$- (Figure 9).

Subsequent to collection of the LC/MS, all sample data were screened for suspected metabolites manually and automatically for unexpected metabolites using the IntelliExtract™ control-comparison data processing tool. In all cases, there was no evidence of metabolism observed in any of the samples by either method and only the anionic form of the residual parent, H-28548, was detected.

G. Elimination Half-Life ($T_{1/2}$)

(Appendix G)

The elimination half-life ($T_{1/2}$) for H-28548 in male and female rats, following a single oral dose at 30 mg/kg, was estimated to be 3 and 8 hours, respectively.

CONCLUSIONS

Following oral administration of H-28548 in water, 96.6% ± 1.43% and 94.6% ± 8.57% of the administered dose was accounted for in urine (0-12 hours) from male and female rats, respectively. At the conclusion of the study (168 hours post-dose), the total accumulated amount of H-28548 detected in urine was 103% ± 2.73% and 99.8% ± 6.41% of the administered dose for male and female rats, respectively.

Elimination of H-28548 via urine was rapid and accounted for a majority of the administered dose for both male and female rats; negligible levels of H-28548 detected in feces from male (1.35% ± 1.05%) and female rats (0.85% ± 0.58%), were likely contamination from of urine.

Cagewash, which is composed of dried excreta (urine and feces), accounted for 0.98% ± 0.52% and 5.03% ± 5.14% of the administered dose for male and female rats, respectively.

Following oral dosing with H-28548 in water and a 168 hour post-dose collection period, 105.3% ± 2.19% and 105.7% ± 1.42% of the administered dose was recovered from male and female rats, respectively.

Samples of urine evaluated using LC/MS were found to contain only the parent substance, H-28548. This finding, taken with the complete recovery of the administered dose in urine, confirms that H-28548 was rapidly absorbed and eliminated unmetabolized following oral dosing in the rat.

The elimination half-life ($T_{1/2}$) for H-28548 in male and female rats, following a single oral dose at 30 mg/kg, was estimated to be 3 and 8 hours, respectively.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, Iron Mountain Records Management, Wilmington, Delaware, or Quality Associates Incorporated, Fulton, Maryland.

REFERENCES

1. DuPont Haskell (2007). In Vitro Rat Hepatocyte Screen. Unpublished report, DuPont-23460.

2. DuPont Haskell (2008). Repeated Dose Oral Toxicity 7-Day Gavage Study in Rats. Unpublished report, DuPont-24009.

3. DuPont Haskell (2007). Biopersistence and Pharmacokinetic Screen in Rats. Unpublished report, DuPont-24281.

4. DuPont Haskell (2009). Cross-Species Comparison of FRD-902 Plasma Pharmacokinetics in the Rat and Primate Following Intravenous Dosing. Unpublished report, DuPont-17751-1579 RV1.

5. DuPont-Haskell (2008). A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery. Unpublished report, DuPont-24447.

TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

CV - coefficient of variation
NA - not applicable
QC - quality control
SD - standard deviation

Table 1
Rat urine sample fortification QC results for H-28548

Rat Urine Fortification Sample	Fortification Concentration (ng/g)	Average Recovery (%)	CV (%)
Low	400	99	2
Mid	100,000	98	1
High	1,000,000	99	1

Table 2
Rat feces sample fortification QC result for H-28548

Rat Feces Fortification Sample	Fortification Concentration (ng/g)	Average Recovery (%)	CV (%)
Low	250	85	6
Mid	1250	85	3
High	62500	91	4

Table 3
Dosing information

	Males		Females	
	Mean	SD	Mean	SD
Subject weight (g)	247.8	8.15	181.1	4.23
Test substance received (mg)	6.79	0.21	4.93	0.10
Dose (mg/kg bw)	27.4	0.17	27.2	0.16

Table 4
Urine, cumulative percent of dose

Post-Dose Time Point (hours)	Males		Females	
	Mean	SD	Mean	SD
Pre-dose	NA	NA	NA	NA
6	68.6	29.4	87.3	11.6
12	96.6	1.43	94.6	8.57
24	101.2	2.69	96.7	8.82
48	102.4	2.91	98.4	7.46
72	102.8	2.76	99.1	6.92
96	102.9	2.75	99.7	6.48
120	103.0	2.74	99.8	6.44
144	103.0	2.73	99.8	6.41
168	103.0	2.73	99.8	6.41

Table 5
Feces, cumulative percent of dose

Post-Dose Time Point (hours)	Males		Females	
	Mean	SD	Mean	SD
0	NA	NA	NA	NA
6	0.74	1.1	NA	NA
12	1.06	0.96	0.36	0.19
24	1.24	0.98	0.50	0.35
48	1.27	0.98	0.64	0.36
72	1.28	0.98	0.75	0.45
96	1.32	1.01	0.82	0.55
120	1.33	1.03	0.83	0.56
144	1.34	1.04	0.84	0.57
168	1.35	1.05	0.85	0.58

Table 6
Material balance, percent of dose

	Males		Females	
	Mean	SD	Mean	SD
Urine	103.0	2.73	99.8	6.41
Feces	1.35	1.05	0.85	0.58
Cage Wash	0.98	0.52	5.03	5.14
Total	105.3	2.19	105.7	1.42

FIGURES

FIGURES

EXPLANATORY NOTES

ABBREVIATIONS:

QC	-	quality control
cps	-	counts per second
m/z	-	mass-to-charge ratio
min	-	minute

Figure 1
Calibration curve for H-28548

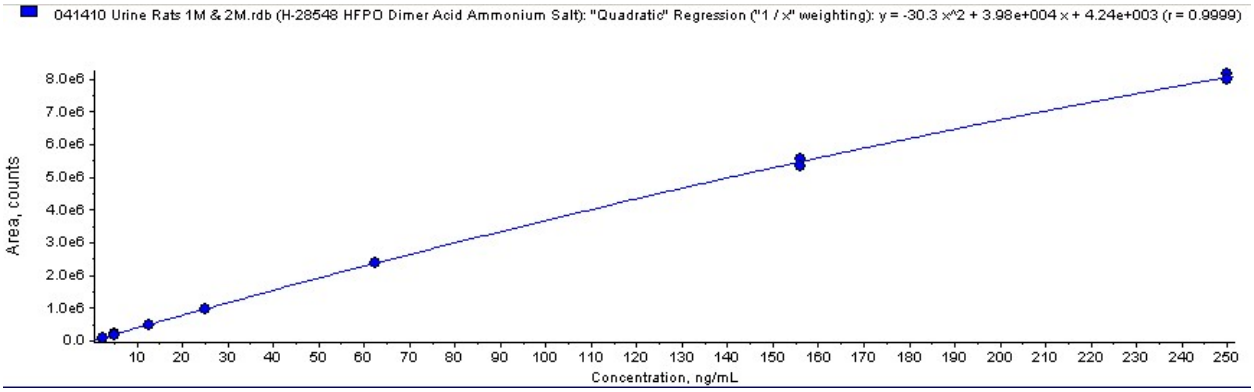
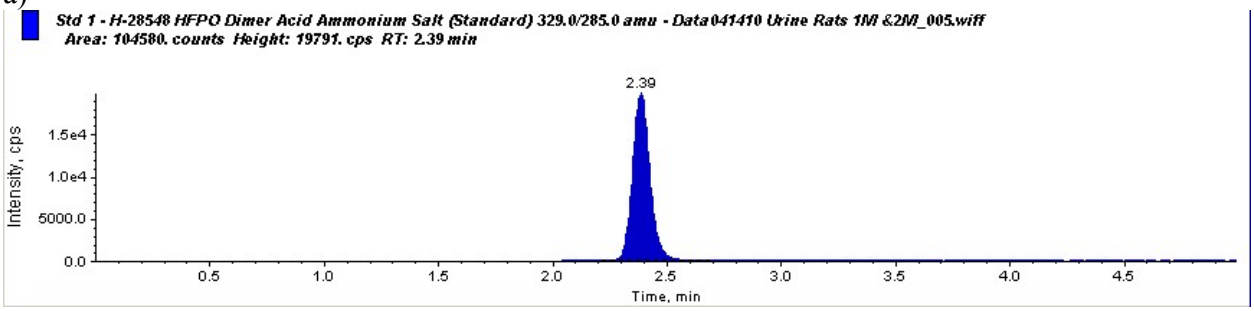


Figure 2
The LC/MS/MS chromatograms for a) lowest calibration standard at 2.5 ng/mL, b) urine control matrix sample, c) low level 400 ng/g fortification QC sample with preparation factor 40x, and d) a 24-hour urine study sample from animal 001M, which had a total dilution factor of 1540x and final concentration of 34700 ng/g

a)



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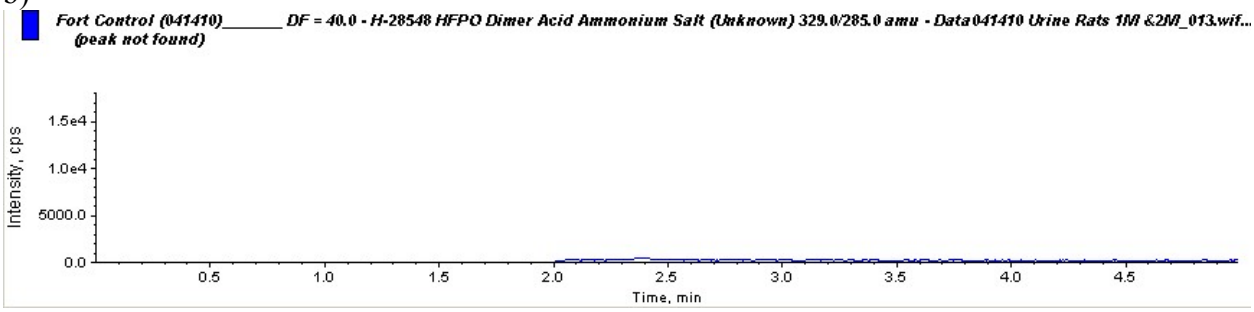

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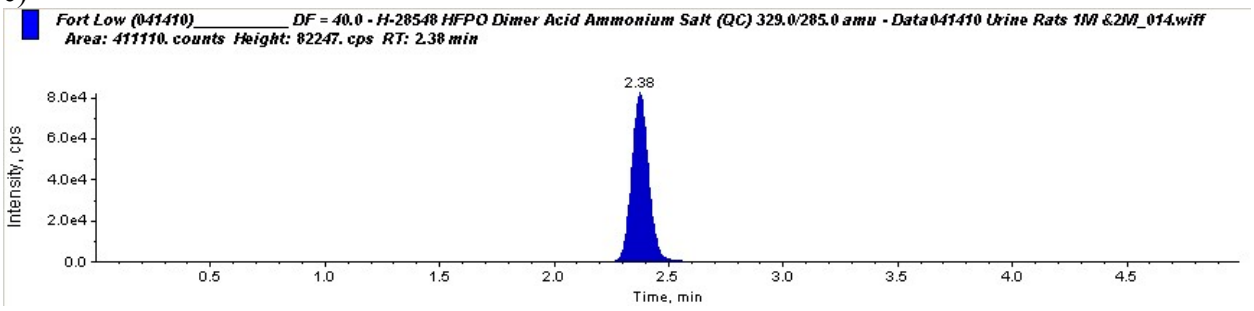

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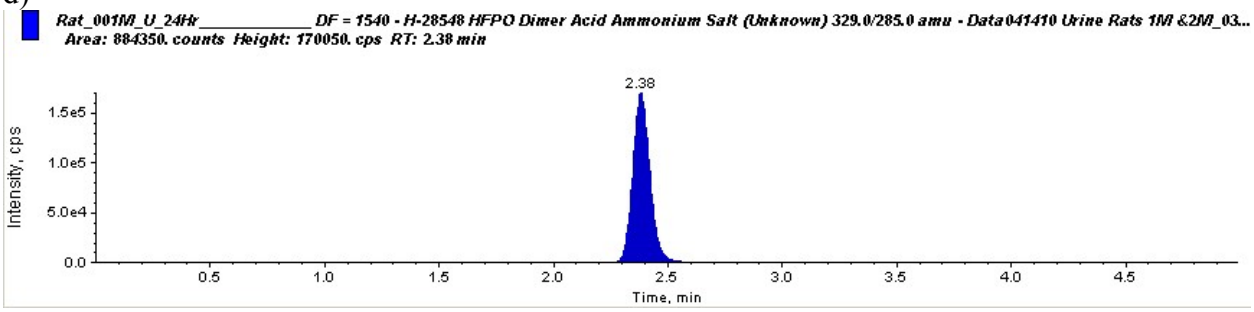
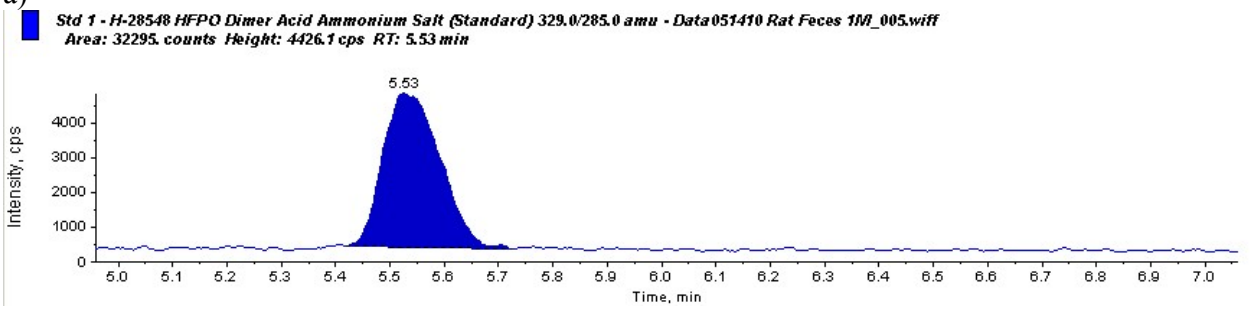


Figure 3
The LC/MS/MS chromatograms for a) lowest calibration standard at 2.5 ng/mL, b) feces control matrix sample, c) low level 250 ng/g fortification QC sample that had a preparation factor of 20x, and d) a 12-hour feces study sample from animal 001M, which had a total 336x dilution factor and final concentration of 2750 ng/g

a)



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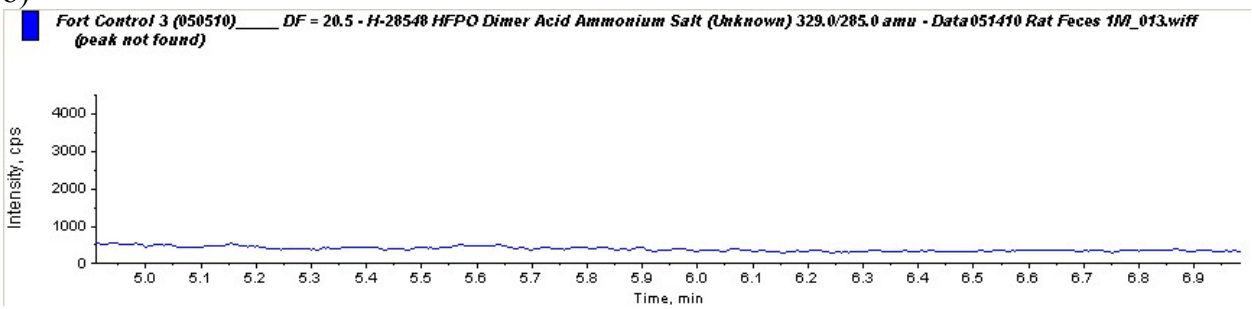

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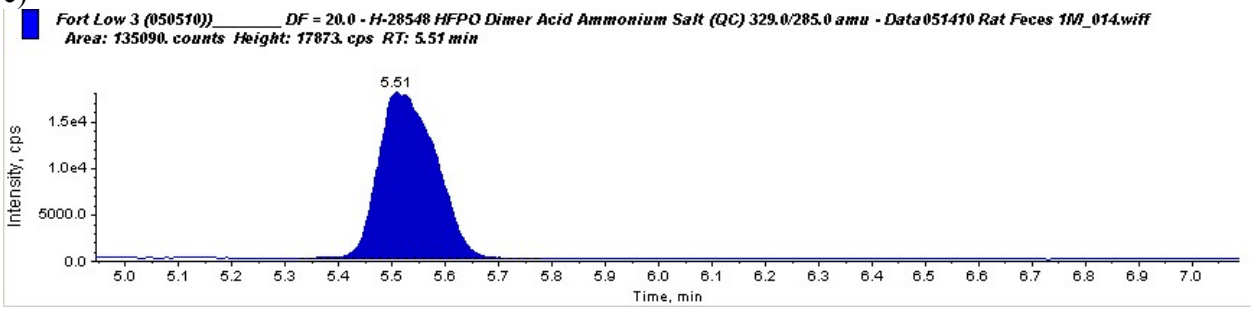

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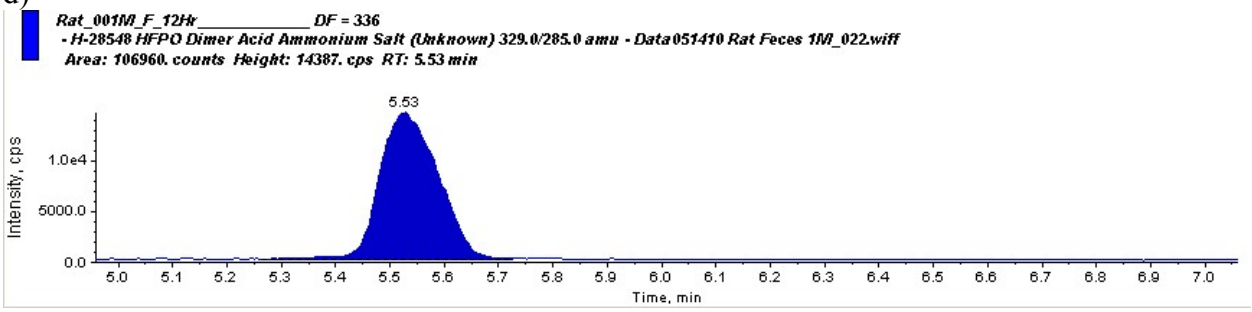


Figure 4
Urine, cumulative percent

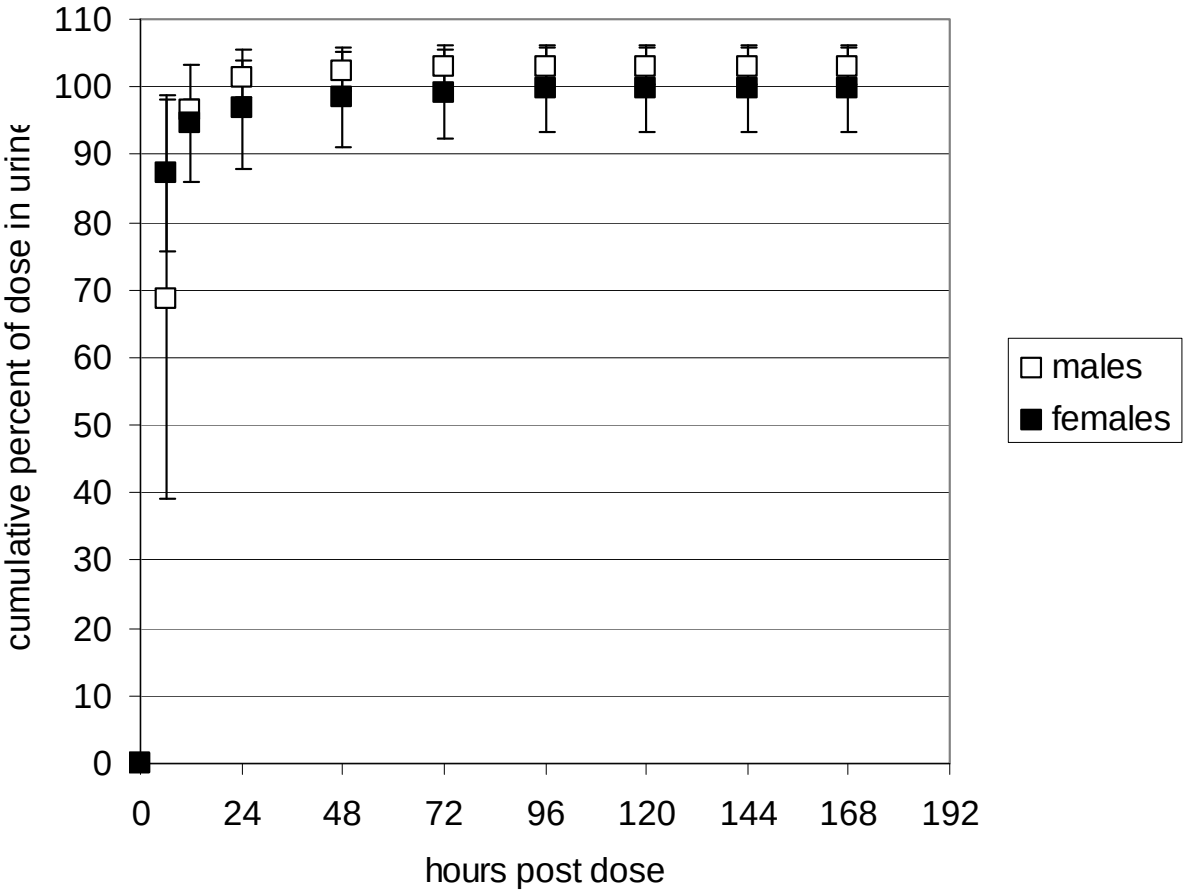


Figure 5
Feces, cumulative percent

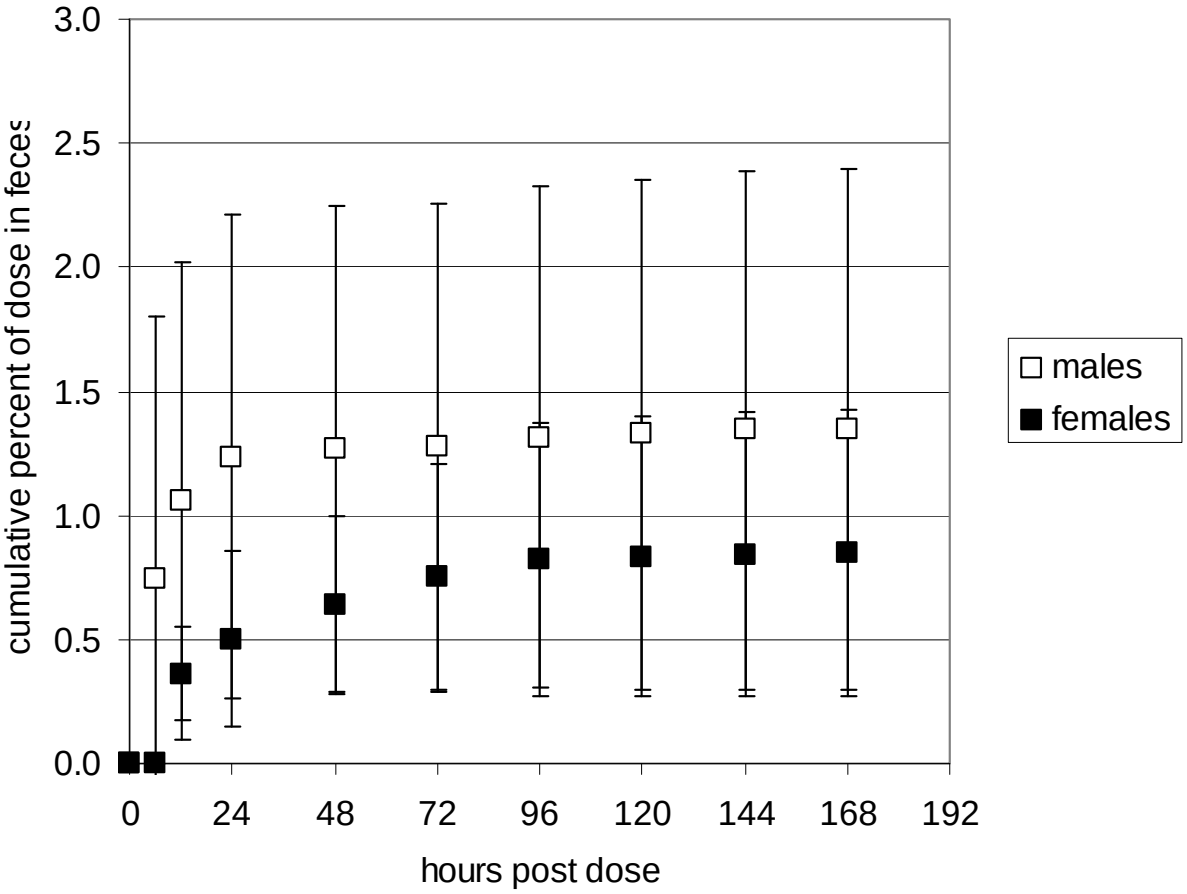


Figure 6
Material Balance, percent of dose

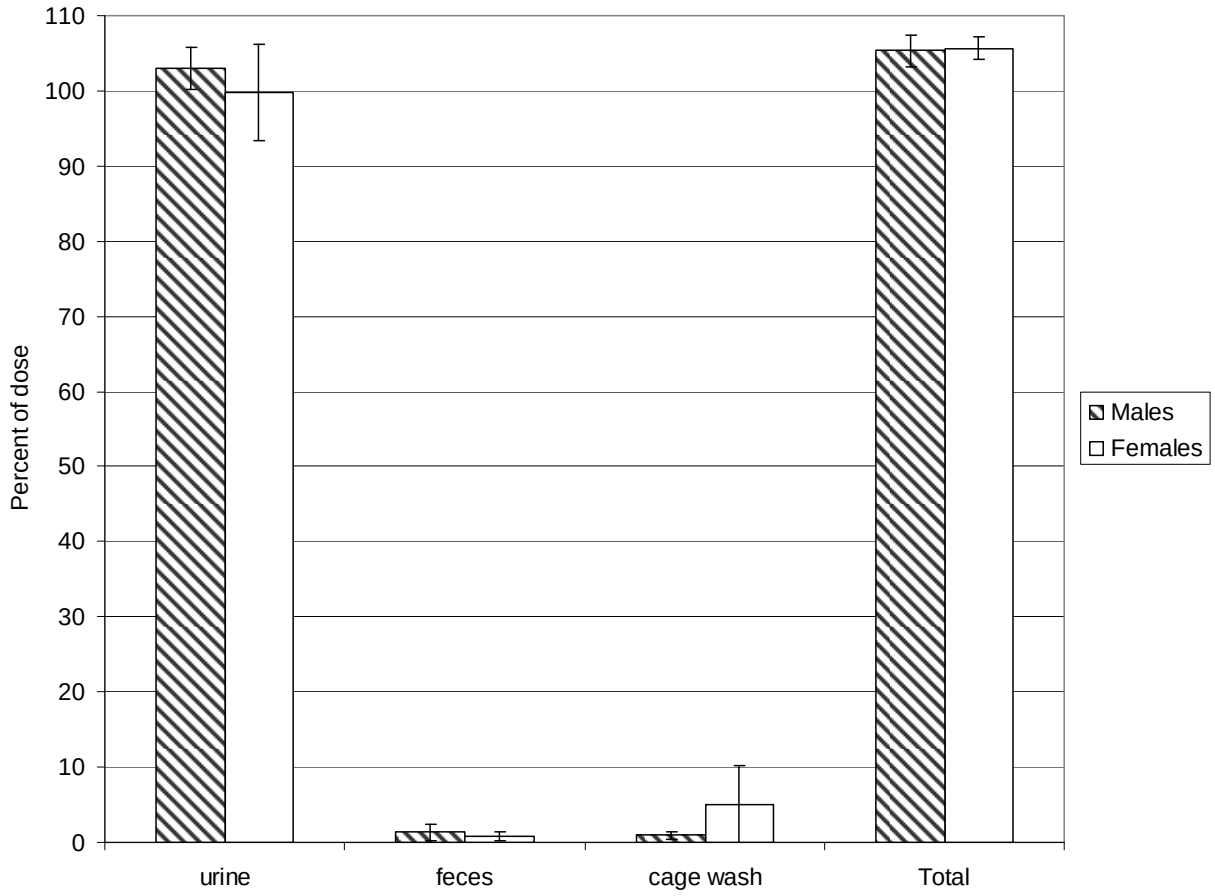


Figure 7
Reconstructed m/z 329 + 659 ion chromatograms characteristic of H-28548-dosed female rat urine (6 hours after administration) – top and control rat urine fortified with H-28458 test substance -bottom

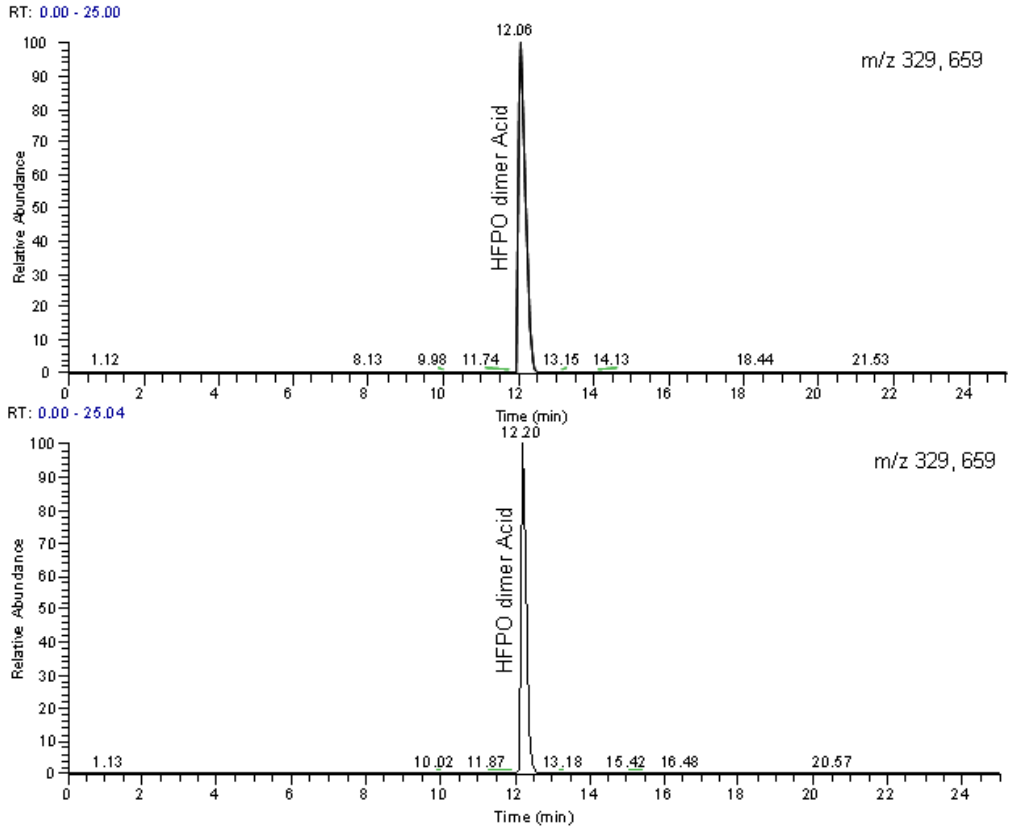


Figure 8
ESI negative mass spectra of H-28548 observed in dosed female rat urine (6 hours after administration)–top; and control urine fortified with H-28548 test substance – bottom

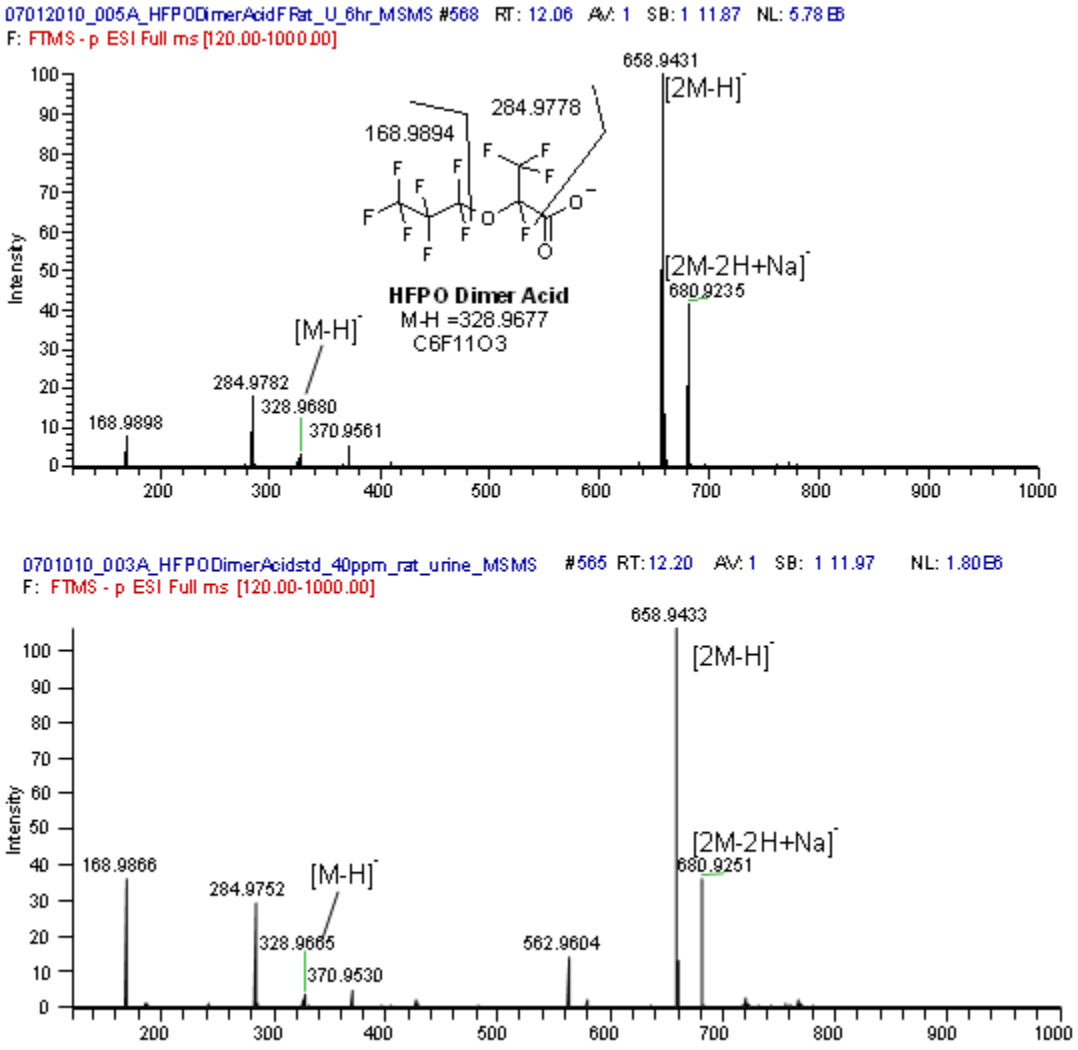
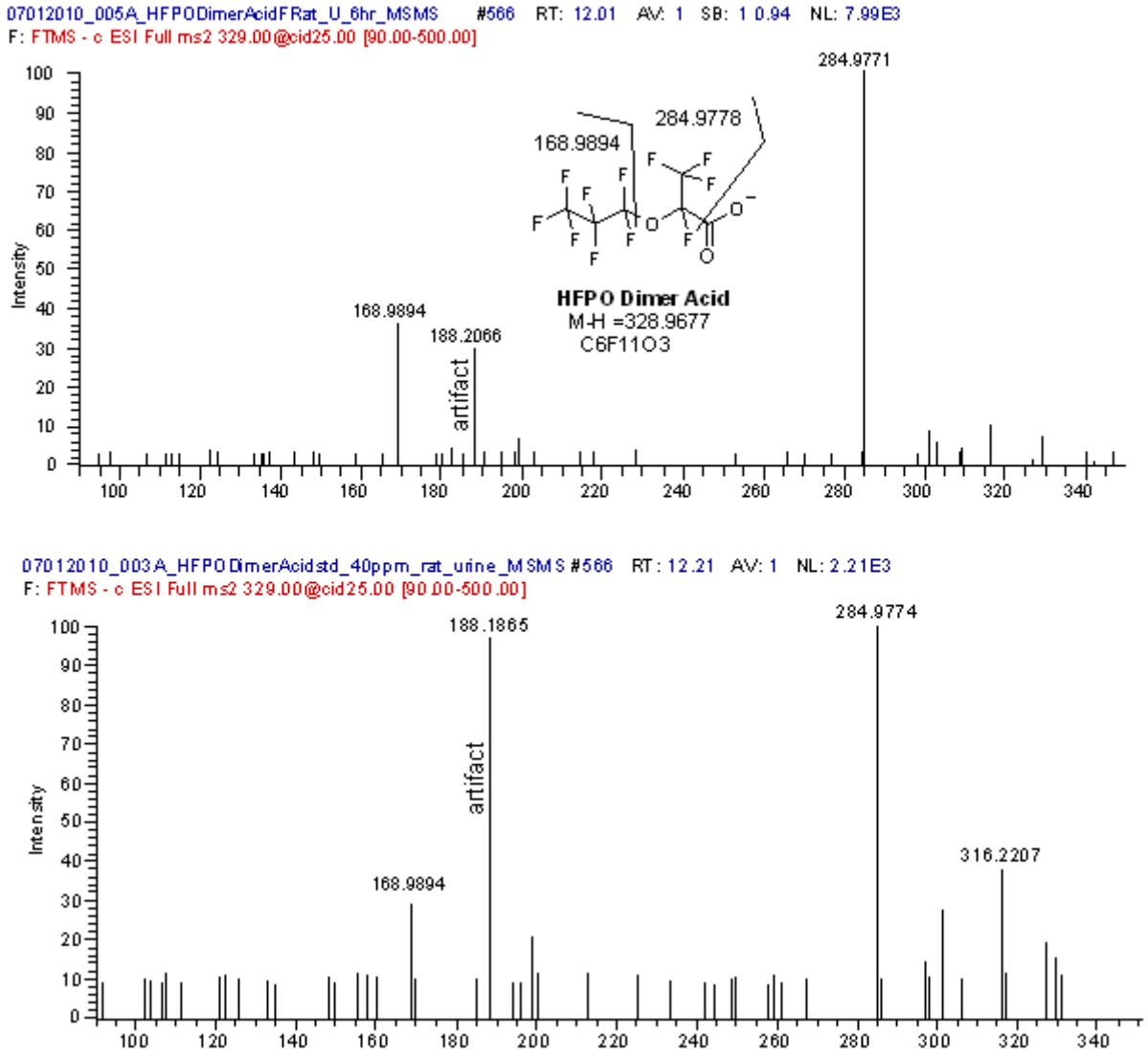


Figure 9
ESI negative daughter ion mass spectra of H28548 observed in dosed female rat urine (6 hours after administration)–top; and control rat urine fortified with H-28548 test substance – bottom



APPENDICES

APPENDICES

EXPLANATORY NOTES

ABBREVIATIONS:

F	-	female
h	-	hours
LOQ	-	limit of quantification
M	-	male
NA	-	not applicable
ND	-	not detected
SD	-	standard deviation

Appendix A
Certificate of Analysis

E. I. du Pont de Nemours and Company
Wilmington, DE 19898
USA

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

Haskell Code Number	H-28548
Common Name	HFPO Dimer Acid Ammonium Salt
Purity Percent	84%
Other Components	Water – 12.7% Perfluorooctanoic acid – 150 ppm
Date of Analysis	June 13, 2008
Expiration Date	June 13, 2011
Instructions for storage	NRT&H
Reference	DuPont-25455
Analysis performed at	E. I. DuPont de Nemours and Company DuPont Haskell Laboratories Newark, Delaware USA

Approver:

Peter A. Bloxham, Ph.D.
Senior Research Chemist

24-JUN-2009
Date

Revision #1: Revised COA expiration date based on compound stability assessment. 6/23/09

Appendix B
Dosing Information

Dosing Information

Males Subject	Subject weight (g)	Compound received (mg)	Dose rate (mg/kg)
001M	247.8	6.78	27.4
002M	241.0	6.56	27.2
003M	255.0	6.97	27.3
004M	256.7	7.01	27.3
005M	238.4	6.60	27.7
Mean	247.8	6.79	27.4
SD	8.15	0.21	0.17

Females Subject	Subject weight (g)	Compound received (mg)	Dose rate (mg/kg)
001F	180.5	4.94	27.4
002F	184.0	5.01	27.2
003F	177.1	4.83	27.3
004F	177.3	4.84	27.3
005F	186.8	5.04	27.0
Mean	181.1	4.93	27.2
SD	4.23	0.10	0.16

Appendix C
Urine Data

Urine Data - Males

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
001M	6782583	Pre-dose	17.314	ND	NA	NA	NA
		6 h	4.837	1180000	5707660	84.2	84.2
		12 h	2.752	301000	828352	12.2	96.4
		24 h	6.358	34700	220623	3.25	99.6
		48 h	22.254	1880	41838	0.62	100.2
		72 h	27.818	2470	68710	1.01	101.2
		96 h	28.961	262	7588	0.11	101.4
		120 h	35.357	<89.5	NA	NA	101.4
		144 h	44.394	<94.3	NA	NA	101.4
		168 h	30.637	<93.3	NA	NA	101.4
						101.4	

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
002M	6564450	Pre-dose	32.306	ND	NA	NA	NA
		6 h	4.228	1250000	5285000	80.5	80.5
		12 h	2.688	417000	1120896	17.1	97.6
		24 h	5.969	35500	211900	3.23	100.8
		48 h	15.124	5270	79703	1.21	102.0
		72 h	10.694	1530	16362	0.25	102.3
		96 h	15.311	544	8329	0.13	102.4
		120 h	44.439	93.7	4164	0.06	102.5
		144 h	43.144	<95.5	NA	NA	102.5
		168 h	37.473	<95.8	NA	NA	102.5
						102.5	

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
003M	6973450	Pre-dose	27.787	ND	NA	NA	NA
		6 h	3.323	1810000	6014630	86.3	86.3
		12 h	2.077	383000	795491	11.4	97.7
		24 h	6.729	55100	370768	5.32	103.0
		48 h	17.212	4470	76938	1.10	104.1
		72 h	16.394	781	12804	0.18	104.3
		96 h	23.082	213	4916	0.07	104.3
		120 h	17.137	<96.5	NA	NA	104.3
		144 h	23.439	<99.3	NA	NA	104.3
		168 h	15.733	<89.5	NA	NA	104.3
						104.3	

Urine Data - Males

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
004M	7007533	Pre-dose	38.816	ND	NA	NA	NA
		6 h	4.373	1210000	5291330	75.5	75.5
		12 h	4.77	275000	1311750	18.7	94.2
		24 h	11.983	21800	261229	3.73	98.0
		48 h	23.195	3890	90229	1.29	99.2
		72 h	25.345	729	18477	0.26	99.5
		96 h	22.124	756	16726	0.24	99.7
		120 h	21.971	143	3142	0.04	99.8
		144 h	21.943	101	2216	0.03	99.8
		168 h	16.324	<95.8	NA	NA	99.8
						99.8	

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
005M	6598533	Pre-dose	14.027	ND	NA	NA	NA
		6 h	2.27	479000	1087330	16.48	16.48
		12 h	4.264	1250000	5330000	80.78	97.3
		24 h	5.469	90400	494398	7.49	104.7
		48 h	16.676	6630	110562	1.68	106.4
		72 h	22.14	691	15299	0.23	106.7
		96 h	20.349	663	13491	0.20	106.9
		120 h	16.363	<87.5	NA	NA	106.9
		144 h	22.415	<94.3	NA	NA	106.9
		168 h	19.651	<93.3	NA	NA	106.9
						106.9	

Timepoint (hours)	Cumulative Mean	SD
0 h	NA	NA
6 h	68.6	29.4
12 h	96.6	1.43
24 h	101.2	2.69
48 h	102.4	2.91
72 h	102.8	2.76
96 h	102.9	2.75
120 h	103.0	2.74
144 h	103.0	2.73
168 h	103.0	2.73

Urine Data - Females

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
001F	4942083	Pre-dose	13.129	ND	NA	NA	NA
		6 h	2.268	1600000	3628800	73.4	73.4
		12 h	2.657	468000	1243476	25.2	98.6
		24 h	6.746	34600	233412	4.72	103.3
		48 h	14.826	3400	50408	1.02	104.3
		72 h	16.819	1290	21697	0.44	104.8
		96 h	19.122	567	10842	0.22	105.0
		120 h	11.956	230	2750	0.06	105.0
		144 h	26.05	142	3699	0.07	105.1
		168 h	19.482	<100	NA	NA	105.1
						105.1	

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
002F	5010250	Pre-dose	14.268	ND	NA	NA	NA
		6 h	2.424	1800000	4363200	87.1	87.1
		12 h	1.805	54100	97651	1.9	89.0
		24 h	7.05	7630	53792	1.07	90.1
		48 h	13.206	8310	109742	2.19	92.3
		72 h	8.605	3240	27880	0.56	92.9
		96 h	19.158	4300	82379	1.64	94.5
		120 h	14.669	820	12029	0.24	94.7
		144 h	20.706	285	5901	0.12	94.9
		168 h	16.431	<105	NA	NA	94.9
						94.9	

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
003F	4826200	Pre-dose	16.215	ND	NA	NA	NA
		6 h	2.807	1350000	3789450	78.5	78.5
		12 h	2.866	63500	181991	3.8	82.3
		24 h	5.164	20400	105346	2.18	84.5
		48 h	13.609	14400	195970	4.06	88.5
		72 h	15.306	5910	90458	1.87	90.4
		96 h	19.344	1360	26308	0.55	91.0
		120 h	13.411	247	3313	0.07	91.0
		144 h	12.458	184	2292	0.05	91.1
		168 h	16.058	<94.3	NA	NA	91.1
						91.1	

Urine Data - Females

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
004F	4839833	Pre-dose	19.326	ND	NA	NA	NA
		6 h	4.46	1070000	4772200	98.6	98.6
		12 h	2.937	38200	112193	2.3	100.9
		24 h	9.506	8310	78995	1.63	102.6
		48 h	23.155	1130	26165	0.54	103.1
		72 h	21.058	870	18320	0.38	103.5
		96 h	27.669	377	10431	0.22	103.7
		120 h	29.855	168	5016	0.10	103.8
		144 h	32.112	<95.8	NA	NA	103.8
		168 h	31.889	<97.0	NA	NA	103.8
						103.8	

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
005F	5037517	Pre-dose	14.453	ND	NA	NA	NA
		6 h	3.101	1610000	4992610	99.11	99.11
		12 h	3.072	48400	148685	2.95	102.1
		24 h	5.328	9410	50136	1.00	103.1
		48 h	18.573	2490	46247	0.92	104.0
		72 h	17.462	549	9587	0.19	104.2
		96 h	18.381	199	3658	0.07	104.2
		120 h	18.371	<90.3	NA	NA	104.2
		144 h	17.949	<96.5	NA	NA	104.2
		168 h	16.373	<92.3	NA	NA	104.2
						104.2	

Timepoint (hours)	Cumulative Mean	SD
0 h	NA	NA
6 h	87.3	11.6
12 h	94.6	8.57
24 h	96.7	8.82
48 h	98.4	7.46
72 h	99.1	6.92
96 h	99.7	6.48
120 h	99.8	6.44
144 h	99.8	6.41
168 h	99.8	6.41

Appendix D
Feces Data

Feces Data - Males

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
001M	6782583	0h	1.976	ND	NA	NA	NA
		6 h	0.986	8240	8125	0.12	0.12
		12 h	4.871	2750	13395	0.20	0.32
		24 h	13.463	935	12588	0.19	0.50
		48 h	11.986	42.7	512	0.01	0.51
		72 h	12.734	46.3	590	0.01	0.52
		96 h	12.325	343	4227	0.06	0.58
		120 h	14.21	<6.95	NA	NA	0.58
		144 h	13.565	97	1316	0.02	0.60
		168 h	12.641	<7.88	NA	NA	0.60
						0.60	

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
002M	6564450	0h	3.741	ND	NA	NA	NA
		6 h	0.192	840000	161280	2.46	2.46
		12 h	2.023	4280	8658	0.13	2.59
		24 h	4.008	2660	10661	0.16	2.75
		48 h	4.893	181	886	0.01	2.76
		72 h	7.026	126	885	0.01	2.78
		96 h	8.513	790	6725	0.10	2.88
		120 h	10.031	321	3220	0.05	2.93
		144 h	11.438	289	3306	0.05	2.98
		168 h	8.604	108	929	0.01	2.99
						2.99	

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
003M	6973450	0h	3.057	ND	NA	NA	NA
		6 h	0.659	3530	2326	0.03	0.03
		12 h	4.431	4240	18787	0.27	0.30
		24 h	7.07	1560	11029	0.16	0.46
		48 h	10.082	96.7	975	0.01	0.47
		72 h	11.949	28.9	345	0.00	0.48
		96 h	10.879	108	1175	0.02	0.50
		120 h	13.489	34.3	463	0.01	0.50
		144 h	12.518	20.6	258	0.00	0.51
		168 h	11.799	<8.50	NA	NA	0.51
						0.51	

Feces Data - Males

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
004M	7007533	0h	6.556	ND	NA	NA	NA
		6 h	0.27	285000	76950	1.10	1.10
		12 h	4.487	4630	20775	0.30	1.39
		24 h	4.822	4050	19529	0.28	1.67
		48 h	10.318	467	4819	0.07	1.74
		72 h	9.184	79.4	729	0.01	1.75
		96 h	10.598	63.7	675	0.01	1.76
		120 h	12.049	30	361	0.01	1.77
		144 h	10.912	15.5	169	0.00	1.77
		168 h	13.156	<7.58	NA	NA	1.77
						1.77	

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
005M	6598533	0h	3.975	ND	NA	NA	NA
		6 h	0.861	150	129	0.002	0.00
		12 h	1.357	33100	44917	0.68	0.68
		24 h	9.349	820	7666	0.12	0.80
		48 h	11.721	258	3024	0.05	0.84
		72 h	9.353	45.3	424	0.01	0.85
		96 h	10.824	27.5	298	0.005	0.86
		120 h	11.997	15.4	NA	NA	0.86
		144 h	10.608	<9.53	NA	NA	0.86
		168 h	11.942	<8.53	NA	NA	0.86
						0.86	

Timepoint (hours)	Cumulative Mean	SD
0 h	NA	NA
6 h	0.74	1.06
12 h	1.06	0.96
24 h	1.24	0.98
48 h	1.27	0.98
72 h	1.28	0.98
96 h	1.32	1.01
120 h	1.33	1.03
144 h	1.34	1.04
168 h	1.35	1.05

Feces Data - Females

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
001F	4942083	0h	2.131	ND	NA	NA	NA
		6 h	NA	NA	NA	NA	NA
		12 h	2.866	4830	13843	0.28	0.28
		24 h	2.097	1360	2852	0.06	0.34
		48 h	6.556	776	5087	0.10	0.44
		72 h	7.927	376	2981	0.06	0.50
		96 h	10.519	152	1599	0.03	0.53
		120 h	8.874	12.1	107	0.00	0.54
		144 h	6.313	50.1	316	0.01	0.54
		168 h	9.804	12.8	NA	NA	0.54
						0.54	

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
002F	5010250	0h	2.05	ND	NA	NA	NA
		6 h	NA	NA	NA	NA	NA
		12 h	4.308	2750	11847	0.24	0.24
		24 h	7.505	475	3565	0.07	0.31
		48 h	9.265	2570	23811	0.48	0.78
		72 h	8.336	1610	13421	0.27	1.05
		96 h	6.43	146	939	0.02	1.07
		120 h	7.961	21	167	0.00	1.07
		144 h	8.581	29	249	0.00	1.08
		168 h	10.028	15	NA	NA	1.08
						1.08	

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
003F	4826200	0h	5.063	ND	NA	NA	NA
		6 h	NA	NA	NA	NA	NA
		12 h	5.571	5950	33147	0.69	0.69
		24 h	6.342	3300	20929	0.43	1.12
		48 h	6.924	590	4085	0.08	1.21
		72 h	10.313	934	9632	0.20	1.40
		96 h	8.19	1640	13432	0.28	1.68
		120 h	9.821	161	1581	0.03	1.72
		144 h	6.169	205	1265	0.03	1.74
		168 h	8.197	92.6	759	0.02	1.76
						1.76	

Feces Data - Females

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
004F	4839833	0h	3.683	ND	NA	NA	NA
		6 h	NA	NA	NA	NA	NA
		12 h	5.018	2270	11391	0.24	0.24
		24 h	3.763	692	2604	0.05	0.29
		48 h	8.256	106	875	0.02	0.31
		72 h	9.595	180	1727	0.04	0.34
		96 h	8.822	76.2	672	0.01	0.36
		120 h	10.684	17.2	184	0.00	0.36
		144 h	7.927	43.6	346	0.01	0.37
		168 h	9.069	12	109	0.00	0.37
						0.37	

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent	Cumulative (%)
005F	5037517	0h	4.772	ND	NA	NA	NA
		6 h	NA	NA	NA	NA	NA
		12 h	5.056	3700	18707	0.37	0.37
		24 h	4.32	945	4082	0.08	0.45
		48 h	8.301	78.3	650	0.01	0.47
		72 h	9.681	48.9	473	0.01	0.47
		96 h	8.19	36.7	301	0.01	0.48
		120 h	8.962	<11.0	NA	NA	0.48
		144 h	8.749	<11.3	NA	NA	0.48
		168 h	7.452	<13.3	NA	NA	0.48
						0.48	

Timepoint (hours)	Cumulative Mean	SD
0 h	NA	NA
6 h	NA	NA
12 h	0.36	0.19
24 h	0.50	0.35
48 h	0.64	0.36
72 h	0.75	0.45
96 h	0.82	0.55
120 h	0.83	0.56
144 h	0.84	0.57
168 h	0.85	0.58

Appendix E
Cage Wash Data

Cage Wash Data - 168 hours

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample Weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent
001M	6782583	168 h	691.966	174	120402	1.78
002M	6564450	168 h	838.827	64	53685	0.82
003M	6973450	168 h	757.65	44	33337	0.48
004M	7007533	168 h	802.957	103	82705	1.18
005M	6598533	168 h	778.34	55	42809	0.65
					Mean	0.98
					SD	0.51

Animal Number	Total H-28548 (ng)	Timepoint (hours)	Sample Weight (g)	Concentration H-28548 (ng/g)	Total Amount (ng H-28548)	Percent
001F	4942083	168 h	798.971	125	99871	2.02
002F	5010250	168 h	977.258	397	387971	7.74
003F	4826200	168 h	1249.369	496	619687	12.84
004F	4839833	168 h	784.33	87	68237	1.41
005F	5037517	168 h	793.16	72	57108	1.13
					Mean	5.03
					SD	5.14

Appendix F
Material Balance

Material Balance

		001M	002M	003M	004M	005M	Mean	SD
urine	6 h	84.2	80.5	86.3	75.5	16.5	68.6	29.4
urine	12 h	12.2	17.1	11.4	18.7	80.8	28.0	29.6
urine	24 h	3.25	3.23	5.32	3.73	7.49	4.60	1.83
urine	48 h	0.62	1.21	1.10	1.29	1.68	1.18	0.38
urine	72 h	1.01	0.25	0.18	0.26	0.23	0.39	0.35
urine	96 h	0.11	0.13	0.07	0.24	0.20	0.15	0.07
urine	120 h	<LOQ	0.06	<LOQ	0.04	<LOQ	0.05	NA
urine	144 h	<LOQ	<LOQ	<LOQ	0.03	<LOQ	0.03	NA
urine	168 h	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	NA	NA
	Subtotal	101.4	102.5	104.3	99.8	106.9	103.0	2.73
feces	6 h	0.12	2.46	0.03	1.10	0.00	0.74	1.06
feces	12 h	0.20	0.13	0.27	0.30	0.68	0.32	0.21
feces	24 h	0.19	0.16	0.16	0.28	0.12	0.18	0.06
feces	48 h	0.01	0.01	0.01	0.07	0.05	0.03	0.03
feces	72 h	0.01	0.01	0.005	0.01	0.01	0.01	0.00
feces	96 h	0.06	0.10	0.02	0.01	0.005	0.04	0.04
feces	120 h	<LOQ	0.05	0.01	0.01	0.003	0.02	0.02
feces	144 h	0.02	0.05	0.004	0.002	<LOQ	0.02	0.02
feces	168 h	<LOQ	0.01	<LOQ	<LOQ	<LOQ	0.01	NA
	Subtotal	0.60	2.99	0.51	1.77	0.86	1.35	1.05
cage wash	168 h	1.78	0.82	0.48	1.18	0.65	0.98	0.52
	Total	103.7	106.3	105.3	102.8	108.4	105.3	2.19

		001F	002F	003F	004F	005F	Mean	SD
urine	6 h	73.43	87.09	78.52	98.60	99.11	87.3	11.6
urine	12 h	25.16	1.95	3.77	2.32	2.95	7.23	10.0
urine	24 h	4.72	1.07	2.18	1.63	1.00	2.12	1.53
urine	48 h	1.02	2.19	4.06	0.54	0.92	1.75	1.43
urine	72 h	0.44	0.56	1.87	0.38	0.19	0.69	0.68
urine	96 h	0.22	1.64	0.55	0.22	0.07	0.54	0.64
urine	120 h	0.06	0.24	0.07	0.10	<LOQ	0.12	0.08
urine	144 h	0.07	0.12	0.05	<LOQ	<LOQ	0.08	0.04
urine	168 h	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	NA	NA
	Subtotal	105.1	94.9	91.1	103.8	104.2	99.8	6.41
feces	6 h	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	NA	NA
feces	12 h	0.28	0.24	0.69	0.24	0.37	0.36	0.19
feces	24 h	0.06	0.07	0.43	0.05	0.08	0.14	0.16
feces	48 h	0.10	0.48	0.08	0.02	0.01	0.14	0.19
feces	72 h	0.06	0.27	0.20	0.04	0.01	0.11	0.11
feces	96 h	0.03	0.02	0.28	0.01	0.01	0.07	0.12
feces	120 h	0.002	0.003	0.03	0.00	<LOQ	0.01	0.01
feces	144 h	<LOQ	0.005	0.03	0.01	<LOQ	0.01	0.01
feces	168 h	<LOQ	0.003	0.02	0.002	<LOQ	0.01	0.01
	Subtotal	0.54	1.08	1.76	0.37	0.48	0.85	0.58
cage wash	168 h	2.02	7.74	12.8	1.41	1.13	5.03	5.14
	Total	107.7	103.7	105.7	105.6	105.8	105.7	1.42

Appendix G
Elimination Half-Life

Elimination Half-Life

OriginLab v7.0220, interpolation of mean urinary excretion data; interpolated data points every 3 hours from 0 to 168 hours(56 data points)

The elimination half-life ($T_{1/2}$) = Cl_{time} (hours) ÷ 6 (elimination half-lives to ≥98.4% of the administered dose)

$T_{1/2}$ Males: Cl_{time} (18 hours) ÷ 6 elimination half-lives = 3 hours
$T_{1/2}$ Females: Cl_{time} (49 hours) ÷ 6 elimination half-lives = 8 hours

Bolded/underlined values (*) identify clearance time (Cl_{time}) to 6 elimination half-lives (≥98.4% of the administered dose) and associated cumulative percent of H-28548 in urine

Cltime (hours)	Cumulative percent of H-28548 eliminated in urine	
	Male	Female
0	40.6	80
3.05455	54.85455	83.71636
6.10909	69.10909	87.43273
9.16364	83.36364	91.14909
12.21818	96.68364	94.63818
15.27273	97.85455	95.17273
18.32727*	***99.02545****	95.70727
21.38182	100.19636	96.24182
24.43636	101.22182	96.73091
27.49091	101.37455	96.94727
30.54545	101.52727	97.16364
33.6	101.68	97.38
36.65455	101.83273	97.59636
39.70909	101.98545	97.81273
42.76364	102.13818	98.02909
45.81818	102.29091	98.24545
48.87273*	102.41455	***98.42545****
51.92727	102.46545	98.51455
54.98182	102.51636	98.60364
58.03636	102.56727	98.69273
61.09091	102.61818	98.78182
64.14545	102.66909	98.87091
67.2	102.72	98.96
70.25455	102.77091	99.04909
73.30909	102.80545	99.13273
76.36364	102.81818	99.20909
79.41818	102.83091	99.28545
82.47273	102.84364	99.36182
85.52727	102.85636	99.43818
88.58182	102.86909	99.51455
91.63636	102.88182	99.59091
94.69091	102.89455	99.66727
97.74545	102.90727	99.70727
100.8	102.92	99.72
103.85455	102.93273	99.73273
106.90909	102.94545	99.74545
109.96364	102.95818	99.75818
113.01818	102.97091	99.77091
116.07273	102.98364	99.78364
119.12727	102.99636	99.79636
122.18182	103	99.8
125.23636	103	99.8
128.29091	103	99.8
131.34545	103	99.8
134.4	103	99.8
137.45455	103	99.8
140.50909	103	99.8
143.56364	103	99.8
146.61818	103	99.8
149.67273	103	99.8
152.72727	103	99.8
155.78182	103	99.8

Cltime (hours)	Cumulative percent of H-28548 eliminated in urine	
	Male	Female
158.83636	103	99.8
161.89091	103	99.8
164.94545	103	99.8
168	103	99.8